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Receptor-mediated uptake of IDL and LDL from nephrotic patients by glomerular epithelial cells

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Receptor-mediated uptake of IDL and LDL from nephrotic patients by glomerular epithelial cells. Although hyperlipidemia is a well-recognized complication of the nephrotic syndrome, the precise interaction of human glomerular cells and human lipoproteins, abnormal in lipid and protein composition, has not been clearly defined. This study examines receptor mediated binding, internalization and degradation as well as intracellular cholesterol metabolism of apoB-100 containing LDL and apoB,E containing IDL, isolated from patients with the nephrotic syndrome (N = 6), in human glomerular epithelial cells and skin fibroblasts. In the patients, serum LDL cholesterol level was increased threefold and IDL elevenfold as compared to healthy subjects. IDL of nephrotic patients contained 72% more cholesterol than IDL of healthy controls. No difference in lipid/protein composition was found in the LDL density range. Therefore, nephrotic and control LDL showed identical affinities for receptor mediated binding, internalization and degradation. Furthermore, inhibition of intracellular sterol synthesis and cholesteryl ester formation after incubation with LDL was comparable. In contrast, cholesterol-rich IDL of nephrotic patients was taken up by glomerular epithelial cells with higher affinity than LDL and control IDL, as well as intracellular sterol synthesis was suppressed more effectively than by control IDL. The cholesterol esterification rate of IDL from patients was enhanced 3.5-fold as compared to control IDL. In comparison to fibroblasts, glomerular epithelial cells showed about 15% of the maximal capacity for LDL uptake, but 31% for IDL from nephrotic patients. The data indicate that hypercholesterolemia of nephrotic origin cannot be explained by reduced ligand binding for LDL. ApoE containing IDL, which accumulate in nephrotic patients, were avidly taken up by glomerular epithelial cells via receptor dependent pathway. These lipoproteins could therefore play the predominant role in glomerular lipid accumulation and development of glomerulosclerosis.

Quantitative abnormalities in lipoprotein metabolism are the most striking features of the nephrotic syndrome. Total serum cholesterol, triglycerides and phospholipids as well as apolipoprotein B, C and E are increased [1]. Qualitative alterations are characterized by cholesterol enrichment in the very-low density lipoprotein (VLDL) fraction as also demonstrated by an increased cholesterol/triglyceride ratio [2, 3]. A similar abnormality has been found in the LDL density range with an enrichment of both, triglyceride and cholesterol [4], but this has not been confirmed by others [1]. Furthermore, in patients with the nephrotic syndrome the IDL fraction is quantitatively increased and characterized by a significant increase of cholesterol at the expense of triglyceride [2]. Increases in serum lipoprotein concentration could be due to increased synthesis [5], impaired catabolism [6], or both [7]. Whether delayed catabolism could also be due to an abnormal composition of lipoproteins or an altered receptor uptake has not yet been studied.

The presence of lipid in the kidney has been described in a wide range of renal disease. In focal and segmental glomerulosclerosis lipid deposition and foam cell formation are almost always present [8]. Moorhead et al [9] hypothesized that accumulation of lipids may injure glomerular cells and predispose to glomerulosclerosis. Further investigations have postulated that glomerulosclerosis and atherosclerosis share common pathogenic mechanisms [10, 11]. Animal experiments have demonstrated that lipid-lowering therapy ameliorates the progression or even suppresses the development of focal and segmental glomerulosclerosis in chronic aminonucleoside nephrosis [12]. Glomerular epithelial cells (GEC) represent the major cell type of the glomerulus in terms of volume [13]. They are directly exposed to high concentrations of lipoproteins in various renal diseases which are accompanied by the nephrotic syndrome. Although characterized by relatively low LDL-receptor activity, GEC clearly exhibit receptor-mediated lipoprotein uptake and high affinities for β-VLDL from cholesterol fed rabbits [14]. Based on these data, we hypothesized that human glomerular cells and skin fibroblasts could exhibit altered receptor mediated uptake and intracellular cholesterol metabolism when exposed to lipoproteins isolated from patients with the nephrotic syndrome. These experiments could further characterize some of the mechanisms by which cellular accumulation of lipids occurs in renal disease.

Methods

Patients

Blood was drawn from six patients (3 female, 3 male, mean age 42 ± 8 years; range 26 to 76 years) with the nephrotic syndrome and serum was isolated for the studies performed *in vitro*. The histologic findings of the patients, as determined by renal biopsy, as well as clinical and laboratory data are shown in Tables 1 and 2. At the time of sampling all patients were

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Table 1. Clinical details of nephrotic patients

Patient	Sex	Age *years*	Weight *kg*	BMI *kg/m²*	Renal histology	Treatment
AE	F	53	62	25.6	MGN	none
JE	M	36	80	24.2	FSGS	frusemide
HC	F	26	46	18.0	MCGN	piretanid, enalapril
ML	F	76	55	22.0	MGN	triamterene, hydrochlorothiazide
EH	M	35	92	29.7	MGN	xipamide
WM	M	26	86	27.8	MGN	frusemide
Mean	3F/3M	42	70	24.6		
SEM		8	7.5	1.7		

Abbreviations are: MGN, membranous glomerulonephritis; FSGS, focal and segmental glomerulosclerosis; MCGN, minimal change glomerulonephritis.

Table 2. Laboratory characteristics of nephrotic patients

Patient	Serum creatinine *mg/dl*	Serum albumin *g/liter*	Quantitative proteinuria *g/24 hr*
AE	0.9	23	8.3
JE	0.8	21	13.0
HC	0.8	19	15.6
ML	0.8	24	8.0
EH	0.9	21	12.3
WM	1.3	17	19.6
Mean	0.9	20.8	12.8
SEM	0.08	1.0	1.8

Table 3. Serum lipid concentrations in patients with the nephrotic syndrome and healthy subjects ($N = 6$)

Subject	Cholesterol *mg/dl*	Triglyceride	VLDL-C	LDL-C	HDL-C
Nephrotic					
AE	578	325	132	401	45
JE	685	620	223	424	38
HC	725	418	141	521	63
ML	574	159	26	429	119
EH	412	319	84	298	30
WM	311	180	46	233	32
Mean	548	337	109	384	55
SEM	65	69	29	42	14
Controls					
Mean	190	81	17	128	45
SEM	10	10	2	7	4

classically nephrotic exhibiting marked edema, severe hypoalbuminemia and hyperlipidemia. No patient was on immunosuppressive or lipid lowering drugs. Informed consent was obtained from all patients. Six normolipidemic healthy subjects, matched for apolipoprotein E phenotype, acted as controls.

Materials

^{125}I-sodium iodide and 2-[^{14}C] sodium acetate were obtained from Amersham-Buchler (Braunschweig, Germany) as well as 9, 10 (n)-[^{3}H] oleic acid from Du Pont (Dreieich, Germany). Collagenase I was purchased from Sigma (Deisenhofen, Germany), and DMEM/F_{12} medium, fetal calf serum (FCS), glutamine, sodium pyruvate, HEPES buffer, penicillin-streptomycin, and non-essential amino acids were all purchased from Seromed (Berlin, Germany). Insulin-transferrin-sodium selenite was obtained from Boehringer-Mannheim (Mannheim, Germany). Rabbit anti-mouse IgG, pig anti-rabbit IgG, peroxidase anti-peroxidase IgG produced in rabbit, antibodies for factor VIII, vimentin and cytokeratin were all from Dakopatts (Hamburg, Germany). Cholesterol, triglycerides and phospholipids were determined enzymatically using Wako reagents (Wako, Neuss, Germany). Antibody to apolipoprotein B was purchased

Table 4. Lipid/protein composition of low density lipoprotein in patients with the nephrotic syndrome and healthy subjects ($N = 6$)

Subject	Chol	TG	PL	Protein	FC	CE
Nephrotic						
AE	40.4	5.6	25.2	28.7	9.2	31.2
JE	57.0	7.6	16.8	18.0	6.8	50.2
HC	39.5	9.8	25.0	25.6	11.4	28.1
ML	49.0	5.5	22.4	22.9	10.6	38.5
EH	42.0	5.1	24.7	29.3	10.0	31.9
WM	35.3	9.0	26.3	28.7	11.1	24.3
Mean	43.8	7.1	23.4	25.4	9.9	34.0
SEM	3.2	0.8	1.4	1.8	0.7	3.7
Controls						
Mean	40.6	5.5	26.8	26.9	11.5	29.2
SEM	0.9	0.7	0.7	1.2	0.9	1.3

Data are given in %. Triglyceride (TG), phospholipid (PL), cholesterol (chol), and protein represent all components of the total lipoprotein. Abbreviations are: FC, free cholesterol; CE, cholesterol ester.

Table 5. Lipid/protein composition of intermediate density lipoprotein in patients with the nephrotic syndrome and healthy subjects ($N = 6$)

Subject	Chol	TG	PL	Protein	FC	CE
Nephrotic						
AE[a]	30.0	19.2	22.0	28.7	8.2	21.7
JE	37.6	21.6	25.0	15.6	9.5	28.0
HC[a]	30.5	29.4	22.0	18.0	9.1	21.4
ML	32.0	28.0	20.0	20.0	8.2	23.8
EH[a]	37.8	21.3	24.5	16.6	10.9	27.0
WM	33.0	24.0	22.7	21.0	8.2	24.8
Mean	33.5[b]	23.9[b]	22.7	20.0[b]	9.0	24.5[b]
SEM	1.4	1.6	0.7	1.9	0.4	1.1
Controls						
Mean	19.2	33.8	19.8	27.6	8.2	12.2
SEM	2.4	1.1	2.1	2.9	0.9	1.6

Data are given in %. Triglyceride (TG), phospholipid (PL), cholesterol (chol), and protein represent all components of the total lipoprotein. Abbreviations are: FC, free cholesterol; CE, cholesterol ester.

[a] Patients studied for IDL uptake

[b] $P < 0.01$ vs. controls

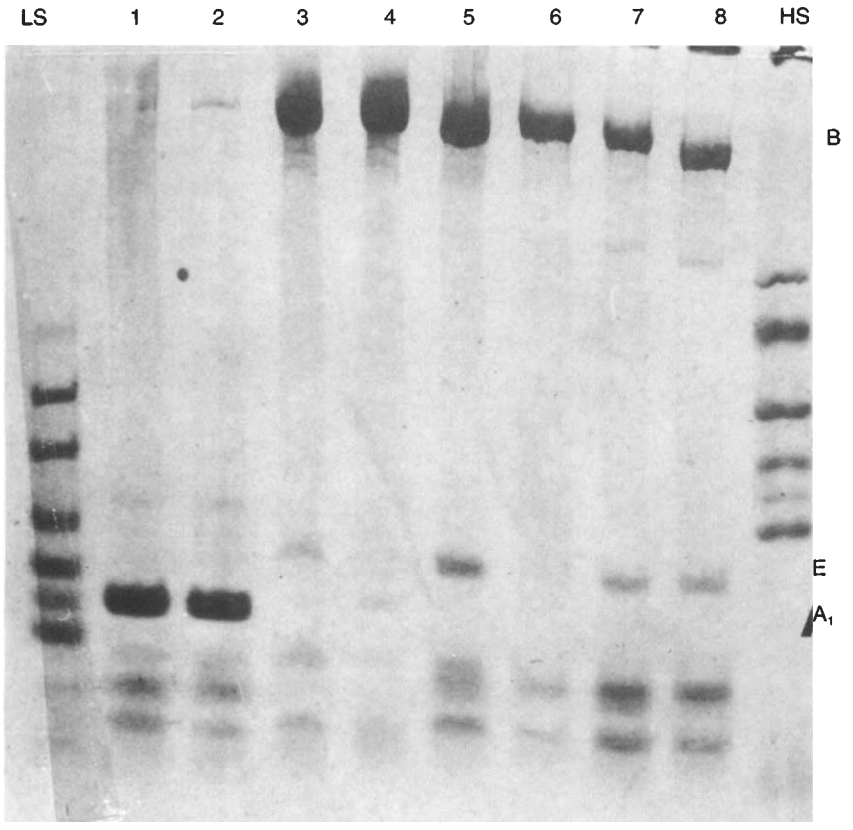

Fig. 1. *Characteristic pattern of apolipoprotein distribution in the various lipoprotein density classes, isolated by ultracentrifugation from a patient with the nephrotic syndrome (3% polyacrylamide gelelectrophoresis in the presence of sodium dodecyl sulfate).* Abbreviations are: LS, low molecular weight standard; 1, HDL nephrotic syndrome; 2, HDL control; 3, LDL nephrotic syndrome; 4, LDL control; 5, IDL nephrotic syndrome; 6, IDL control; 7, VLDL nephrotic syndrome; 8, VLDL control; B, apolipoprotein B-100; E, apolipoprotein E, A1, apolipoprotein A1; HS, high molecular weight standard.

from Beckman (Palo Alto, California, USA) and apolipoprotein E from International Immunology Corporation (Murrista, California, USA).

Cell cultures

Fibroblasts derived from skin biopsies of healthy subjects were maintained in Dulbecco's minimal essential medium, which contained 25 mM $NaHCO_3$, 20 mM HEPES buffer, pH 7.4 and 10% FCS. Kidney tissue was obtained from patients undergoing tumor nephrectomy and glomeruli were isolated by sieving technique as previously described [15]. In brief, isolated glomeruli were incubated with collagenase I for 30 minutes at 37°C. After washing twice in the DMEM/F_{12} medium and following sedimentation cycles, aside from glomeruli almost no other particles could be detected. Encapsulated glomeruli were not observed visually. They were suspended in DMEM/F_{12} culture medium, supplemented with 10% heat-inactivated FCS, glutamine 2.5 mmol/liter, sodium pyruvate 0.1 mmol/liter, HEPES buffer 5 mmol/liter, penicillin 10^5 U/liter, streptomycin 100 mg/liter, non-essential amino acids (100×) 0.1 × and insulin-transferrin-sodium selenite supplement 5 mg/liter. Glomeruli were plated onto Petri dishes coated with collagen (Vitrogen 100^R, Celtrix, Palo Alto, California, USA). Collagen coated plastic material was recently shown to facilitate the outgrowth of glomerular epithelial cells [16]. After one week in primary culture GEC began to sprout around the glomeruli. After destruction of residual mesangial cells with a scalpel blade GEC were subcultured. Fibroblast contamination was excluded by growing the cells for two weeks in a L-valine free medium according to Gilbert and Migeon [17]. Cells from the fifth to the tenth subculture were used for all experiments.

Morphologic and immunochemical studies

Glomerular epithelial cells in culture could be identified with phase-contrast microscopy. They showed an epithelial morphology, a polyhedral shape and a cobblestone-like appearance when confluency was reached. All cell lines showed the same immunological characteristics as described by Kreisberg and Karnowski [18]. Immunochemical characterization was performed using the peroxidase-antiperoxidase (PAP) method according to Bross et al [19]. Confluent cell layers were fixed with methanol at −20°C. Cells were preincubated for 30 minutes with Dulbeccos's phosphate saline buffer (PBS-buffer) containing gelatin 5% and serum albumin 22% to block nonspecific protein binding. The cells were then incubated with a mouse anti-human IgG antibody for 30 minutes. The following "sandwich" antibodies were each incubated for 15 minutes: rabbit anti-mouse IgG, pig anti-rabbit IgG, peroxidase anti-peroxidase IgG produced in rabbit. After staining the immuncomplex with diaminobenzidine, brown granules in PAP-positive cells could be observed by light microscopy. Cells not incubated with the primary antibody failed to show any staining excluding nonspecific reactions. Antibodies for factor VIII did not stain, thus excluding endothelial cells. Cells were homogenously positive for vimentin and cytokeratin, thus excluding mesangial cells. Therefore, the cells studied showed the morphological and immunological characteristics described by others for GEC [20, 21]. At present, it is not possible to determine specifically whether GEC in culture originate from visceral or parietal

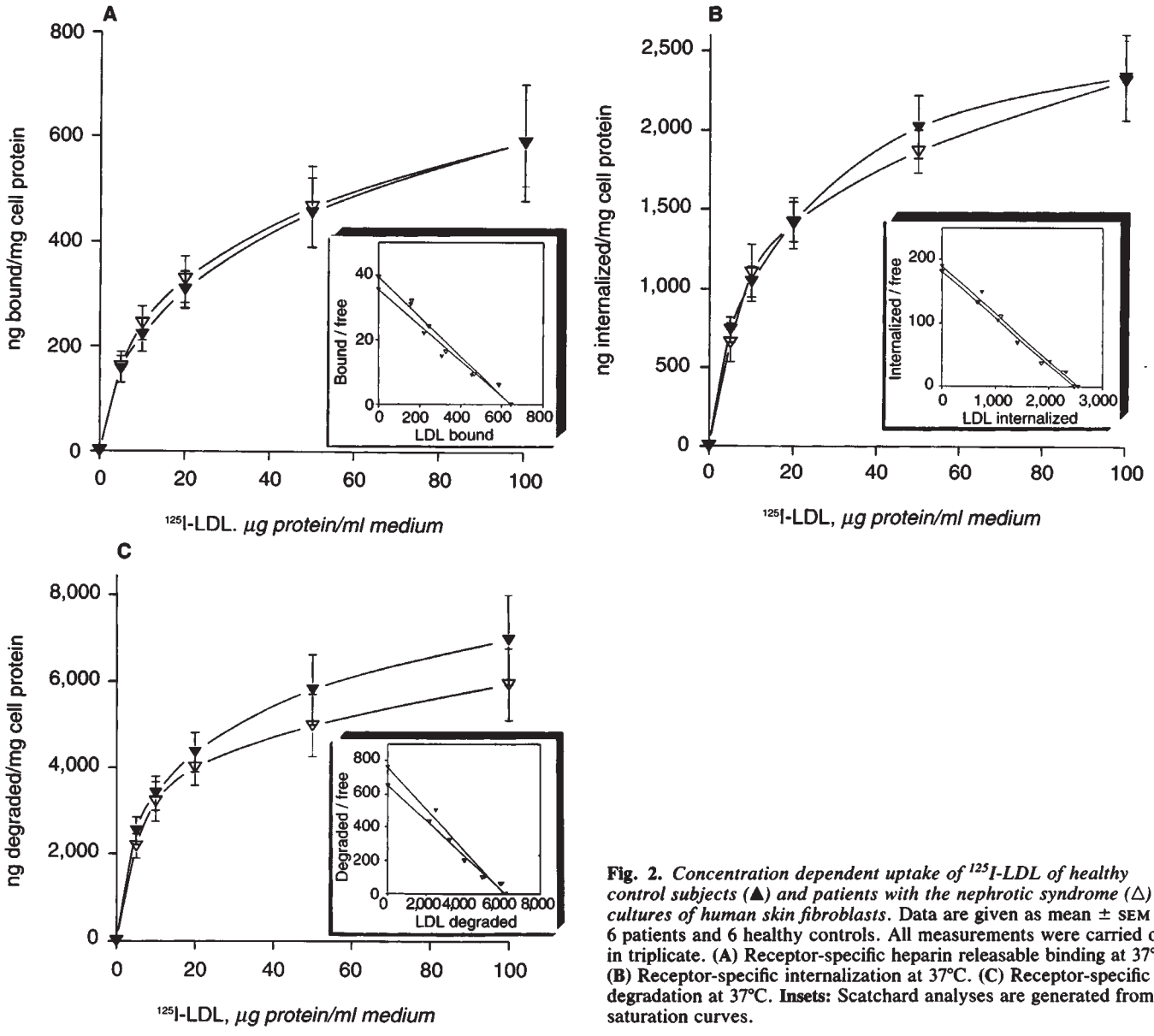

Fig. 2. *Concentration dependent uptake of ^{125}I-LDL of healthy control subjects (▲) and patients with the nephrotic syndrome (△) by cultures of human skin fibroblasts.* Data are given as mean ± SEM of 6 patients and 6 healthy controls. All measurements were carried out in triplicate. **(A)** Receptor-specific heparin releasable binding at 37°C. **(B)** Receptor-specific internalization at 37°C. **(C)** Receptor-specific degradation at 37°C. **Insets:** Scatchard analyses are generated from saturation curves.

epithelium. Cytokeratin negativity and vimentin positivity has been suggested as a criterion for visceral origin [22], but this is not generally accepted [23].

Lipid and apolipoprotein analysis

Very-low density lipoprotein (VLDL) was isolated by analytical ultracentrifugation [24] with modifications previously described [25]. The concentration of LDL-lipids was determined by the difference between VLDL-free serum and the supranate of this fraction after precipitation of LDL by phosphotungstic acid/$MgCl_2$. Cholesterol, triglycerides and phospholipids were determined enzymatically on a Wako R-30 analyzer. The coefficient of variation for cholesterol and triglyceride as well as the determination of LDL varied between 1 and 3%. Apolipoprotein B was determined by kinetic nephelometry using a commercial kit and the "Array" nephelometer (Beckman). Apolipoprotein E was measured by endpoint nephelometry using a "BNA" nephelometer from Behring (Marburg, Germany). The specificity of antiserum was evaluated by immunoelectrophoresis for apoB and immunofixation in agarose for apoE. Apolipoprotein E phenotyping was performed by immunofixation in agarose as described previously [26]. The apolipoprotein composition of the VLDL, IDL and LDL fraction was further characterized by SDS-PAGE on 3% polyacrylamide slab gels [27]. The protein content of cells and lipoproteins was determined by the Lowry method.

Lipoprotein isolation and separation

Blood from the nephrotic patients as well as the six healthy donors was drawn into tubes containing 10% sodium-citrate

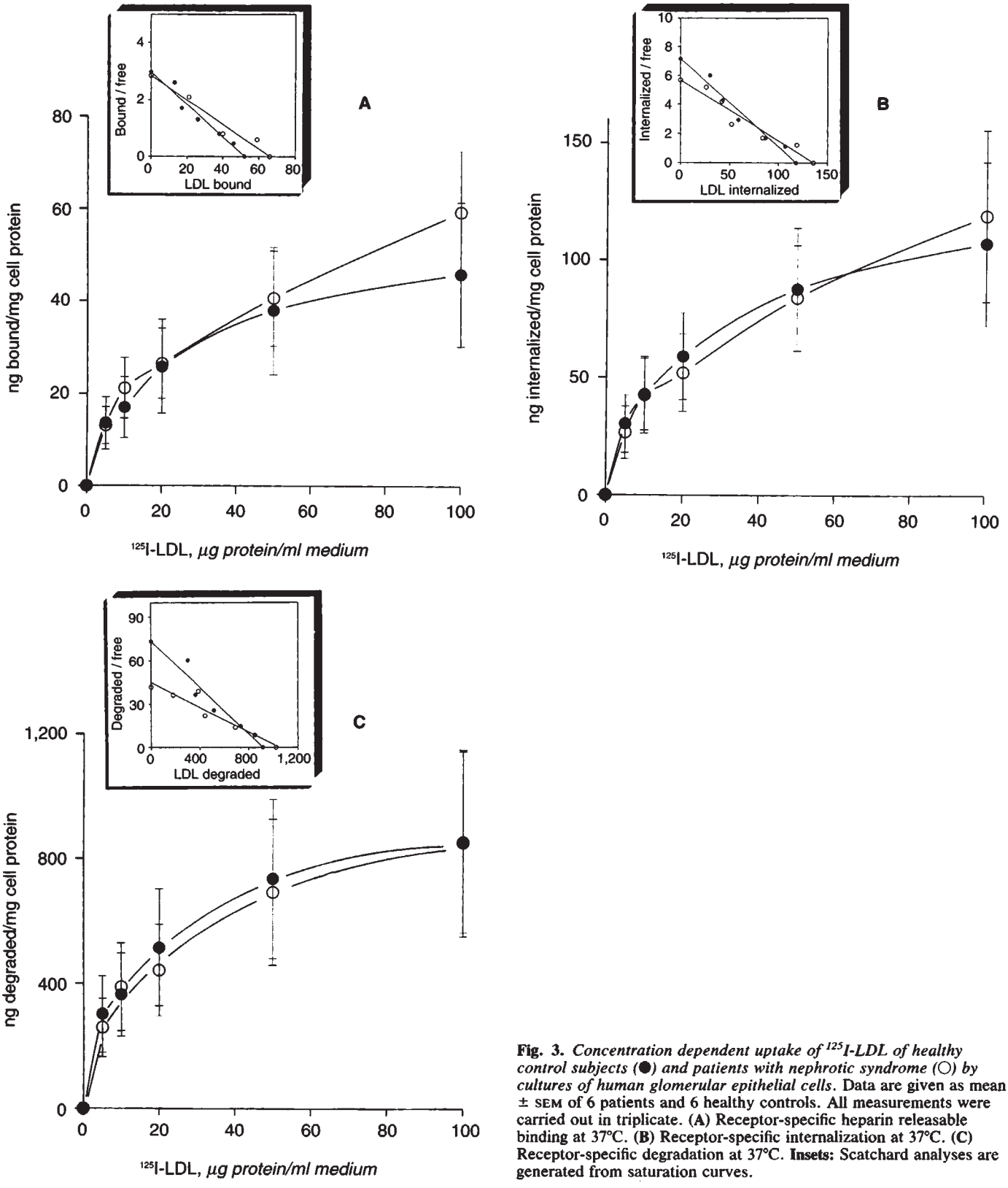

Fig. 3. *Concentration dependent uptake of ^{125}I-LDL of healthy control subjects (●) and patients with nephrotic syndrome (○) by cultures of human glomerular epithelial cells.* Data are given as mean ± SEM of 6 patients and 6 healthy controls. All measurements were carried out in triplicate. (**A**) Receptor-specific heparin releasable binding at 37°C. (**B**) Receptor-specific internalization at 37°C. (**C**) Receptor-specific degradation at 37°C. **Insets:** Scatchard analyses are generated from saturation curves.

after a 12-hour overnight fast. Lipoproteins of patients were isolated in parallel with the control subjects by sequential ultracentrifugation [28]. After separation, VLDL, IDL and LDL were dialyzed against 5 mM Tris/HCl pH 7.4, 154 mM NaCl, 250 mM EDTA, and sterilized by passage through a 0.45 µm Millipore filter.

Table 6. Concentration dependent uptake of ^{125}I-IDL and ^{125}I-LDL from patients with the nephrotic syndrome by monolayer cultures of human glomerular epithelial cells (GEC) and skin fibroblasts (FIB)

	GEC		FIB	
	V_{max}	K_d	V_{max}	K_d
Control LDL				
Specific binding	66	23.4	644	16.4
Specific internalization	135	23.8	2,476	13.6
Specific degradation	1,024	21.4	6,181	9.5
Nephrotic LDL				
Specific binding	52	17.6	646	18.2
Specific internalization	118	16.5	2,551	13.5
Specific degradation	916	12.4	6,098	8.0
Control IDL				
Specific binding	38	16.5	191	26.9
Specific internalization	158	18.1	1,248	28.3
Specific degradation	295	7.3	2,229	11.4
Nephrotic IDL				
Specific binding	62	7.4	180	3.4
Specific internalization	194	8.3	1,744	6.1
Specific degradation	1,073	5.1	2,240	2.4

Mean ± SEM from 6 LDL experiments and 3 IDL experiments. All measurements were done in triplicate. Data are obtained by scatchard plot analysis. V_{max} is given in ng/mg cell protein and K_d in μg/ml medium.

Preparation of lipoprotein deficient serum (LDS)

Human plasma was rendered lipoprotein deficient by a two step ultracentrifugation procedure (150,000 × g for 48 hr at 10°C), adjusting the plasma to d = 1.250 g/ml by addition of solid KBr. After removal of all lipoprotein fractions LDS was dialyzed against 154 mM NaCl, 250 mM EDTA, 5 mM HEPES, pH 7.4. Thereafter, LDS was heat-inactivated at 54° for one hour and sterilized by passage through a 0.45 μm Millipore filter. Finally the protein content was adjusted to 40 mg/ml.

Radioiodination of IDL and LDL

Radioiodination of IDL and LDL was performed by the method of McFarlane [29] modified for lipoproteins by Bilheimer, Eisenberg and Levy [30]. Per mg lipoprotein-protein 100 μl glycine buffer (1 M, pH 10), 25 mCi ^{125}I-sodium iodide and 30 μl iodine-monochloride (10 mM) were added. Most unbound iodine was removed by passage through a Sephadex G-25 column and by dialyzing against buffer containing 154 mM NaCl, 250 mM EDTA pH 7.4. The final preparations of ^{125}I-IDL and ^{125}I-LDL were sterilized by passage through a 0.45 μm Millipore filter and protein content was estimated by using Lowry method.

Assay for binding, internalization and degradation of lipoproteins

To induce maximal apo B,E receptor activity, GEC were incubated in medium that contained 10% LDS instead of FCS. After 48 hours, monolayers were washed with PBS, and fresh LDS containing medium was added together with ^{125}I-labeled lipoproteins with or without a 25-fold excess of unlabeled lipoproteins. The cultures were incubated for five hours at 37°C [31]. The medium was removed and the cells were placed on ice. The culture dishes were then washed five times with a cold buffer containing 154 mM NaCl, 50 mM Tris (pH 7.4) and 2 mg/ml bovine fatty acid-free serum albumin as described by Goldstein, Kita and Brown [31]. Assays for binding, internalization and degradation were performed by standard methods [32]. The heparin releasable activity represents binding at 37°C. After the release of cell surface bound activity by heparin, monolayers were dissolved in 1 ml of 0.1 N NaOH and radioactivity and protein were measured. The radioactivity in the pellet was taken as a measure for internalization. Non-iodide trichloroacetic acid (TCA)-soluble radioactivity served as a measure for lipoprotein degradation. Non-specific binding, internalization and degradation was defined as amount of the lipoprotein taken up in the presence of 25-fold excess of unlabeled ligand. All experiments were performed in triplicate.

Cellular sterol synthesis and cholesterol esterification

Cell cultures were incubated in 10% LDS containing DMEM medium for 48 hours and thereafter with varying concentrations of lipoproteins for 12 hours.

Cellular sterol synthesis. Incorporation of [^{14}C] acetate into sterols was used to determine cellular sterol synthesis. After preincubation one ml of medium containing 0.8 μCi of [^{14}C] acetate and unlabeled carrier acetate (0.2 μmol/ml, specific acitivity 4 μCi/μmol) was added to each dish and the incubations were continued for two hours at 37°C. After washing twice with PBS/BSA medium and three times with PBS-buffer, cells were dissolved in 2 ml of 0.1 N NaOH. Cell suspension was ultrasonified and a portion was taken for determination of [^{14}C] activity and protein content [30, 31]. One ml of 100% ethanol and 0.2 ml of 90% KOH were added in order to saponify cell lysate during three hours at 80°C. Non-saponifiable lipids were extracted into 2.5 ml of hexane. The hexane layer was washed once by the addition of 0.1 mM sodium acetate (2.5 ml). The hexane phase was evaporated and [^{14}C] activity was measured. All experiments were performed in duplicate.

Cellular cholesterol esterification. Cellular cholesterol esterification was performed as described elsewhere [14]. Preincubation was done as described above. LDS medium containing 1 μCi of 9, 10 (n)-[^{3}H] oleic acid bound to fatty acid-free albumin in a molar ratio of 1:4 was added to monolayer cultures (specific activity 20 μCi/μmol and final [^{3}H] oleate concentration of 50 nmol/ml). After two hours of incubation at 37°C, cells were then washed with PBS, scraped into plastic tubes and pelleted by centrifugation. Water (0.4 ml) was added and pellets were homogenized by ultrasonification. An aliquot (0.2 ml) of homogenate was extracted with 25 volumes of chloroform/methanol (2:1), and the chloroform phase was washed with 0.034% $MgCl_2$ (0.2 ml/ml of chloroform/methanol extract). After washing, the chloroform phase was dried, resuspended in chloroform/methanol and chromatographed on silica gel thin-layer plates using a solvent system hexane:diethylether:acetic acid (83:16:1, vol/vol/vol). Ester bands comigrating with cholesteryl oleate standard were scraped and [^{3}H] oleate incorporated in cholesteryl ester quantitated. The water homogenate of cultures was used for measurement of cellular protein as well as direct measurement of [^{3}H] activity. All experiments were performed in duplicate.

Statistical evaluation

Results are given as means ± SEM. The means of the duplicate and triplicate determinations from each experiment served as a single experiment. Comparison was performed by paired or unpaired Student's *t*-test, as appropriate.

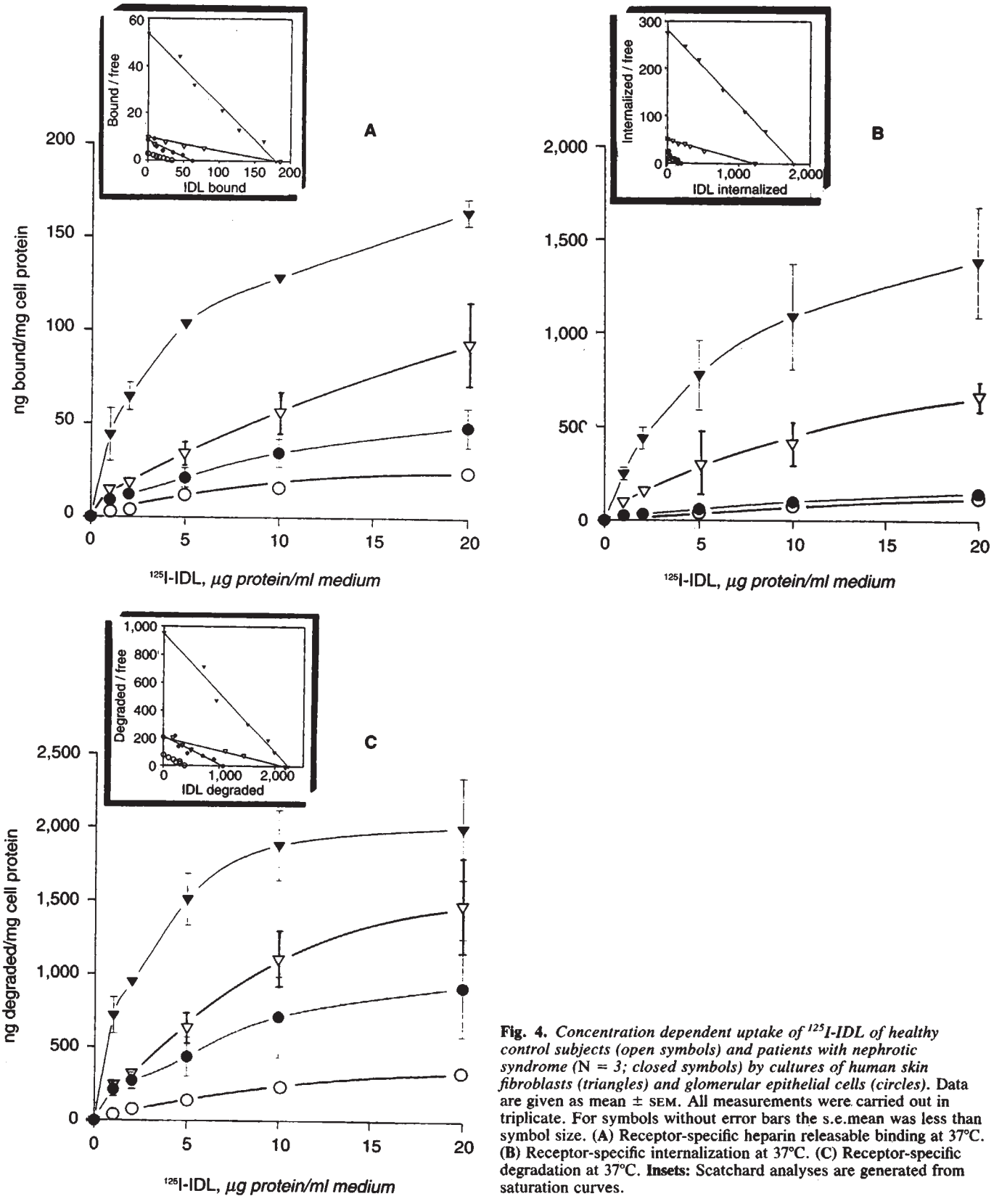

Fig. 4. *Concentration dependent uptake of ^{125}I-IDL of healthy control subjects (open symbols) and patients with nephrotic syndrome (N = 3; closed symbols) by cultures of human skin fibroblasts (triangles) and glomerular epithelial cells (circles).* Data are given as mean ± SEM. All measurements were carried out in triplicate. For symbols without error bars the s.e.mean was less than symbol size. (**A**) Receptor-specific heparin releasable binding at 37°C. (**B**) Receptor-specific internalization at 37°C. (**C**) Receptor-specific degradation at 37°C. **Insets:** Scatchard analyses are generated from saturation curves.

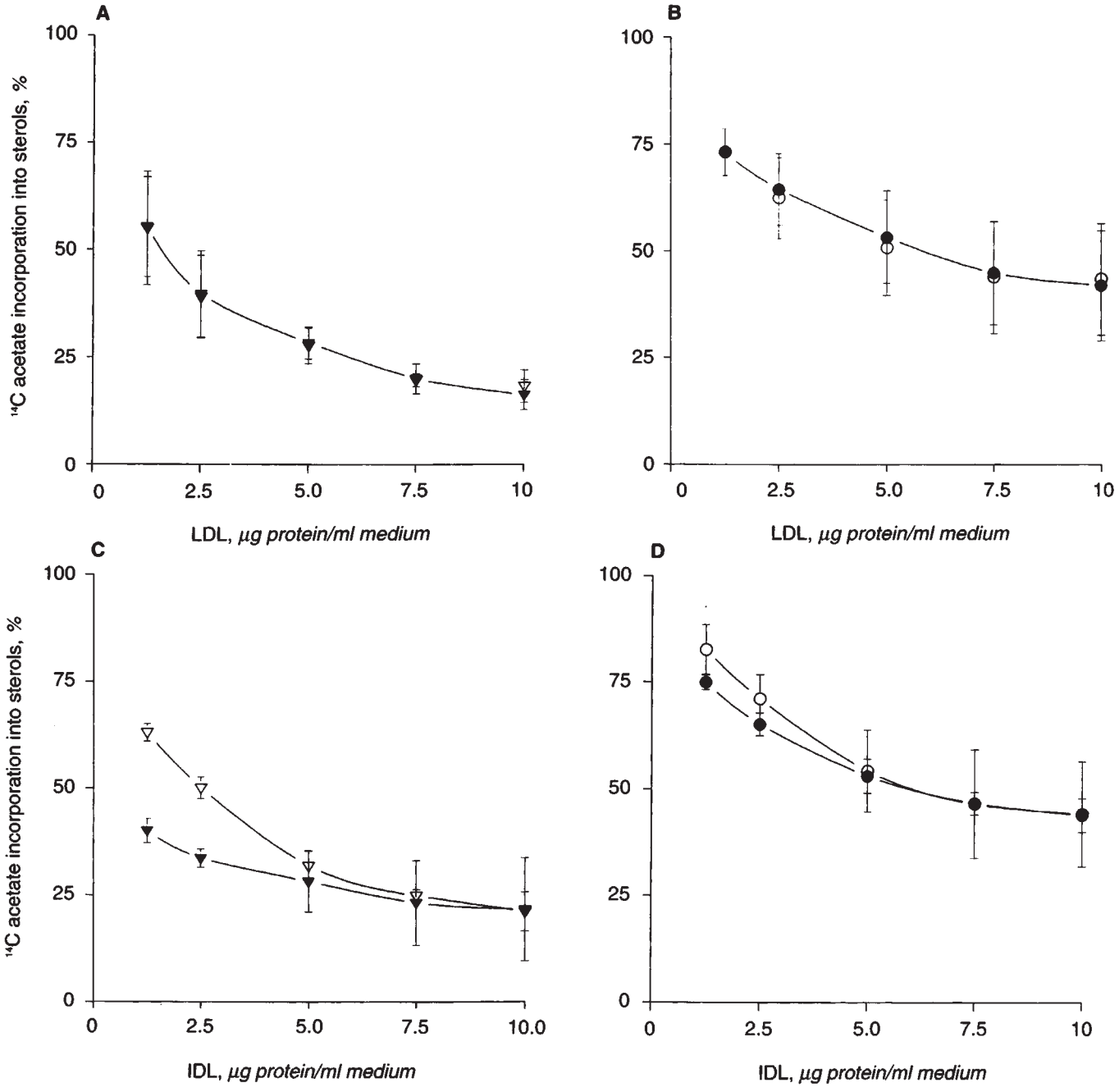

Fig. 5. *Inhibition of cholesterol synthesis by IDL and LDL of healthy subjects (open symbols) and patients with the nephrotic syndrome (closed symbols) in human skin fibroblasts (triangles) and human glomerular epithelial cells (circles).* Incorporation of [^{14}C] acetate into sterols is expressed in picomol of acetate found in non-saponifiable lipids. Data are given as mean of three independent determinations. All measurements were carried out in duplicate.

Results

Serum lipid concentration in nephrotic patients

Absolute values for serum lipids of patients with the nephrotic syndrome as well as healthy subjects are given in Table 3. The patients exhibited 2.9-fold higher concentrations of serum cholesterol and markedly higher levels of serum triglycerides. Levels of VLDL cholesterol and LDL cholesterol in nephrotic patients were also higher than those in healthy subjects, but HDL cholesterol was not different. Both, patients and healthy subjects were matched for apoE3/E3 phenotype.

Lipid/protein composition of IDL and LDL from nephrotic patients

To investigate whether elevated serum lipids in nephrotic patients were also accompanied by an abnormal lipoprotein composition, the lipid/protein content of IDL and LDL was

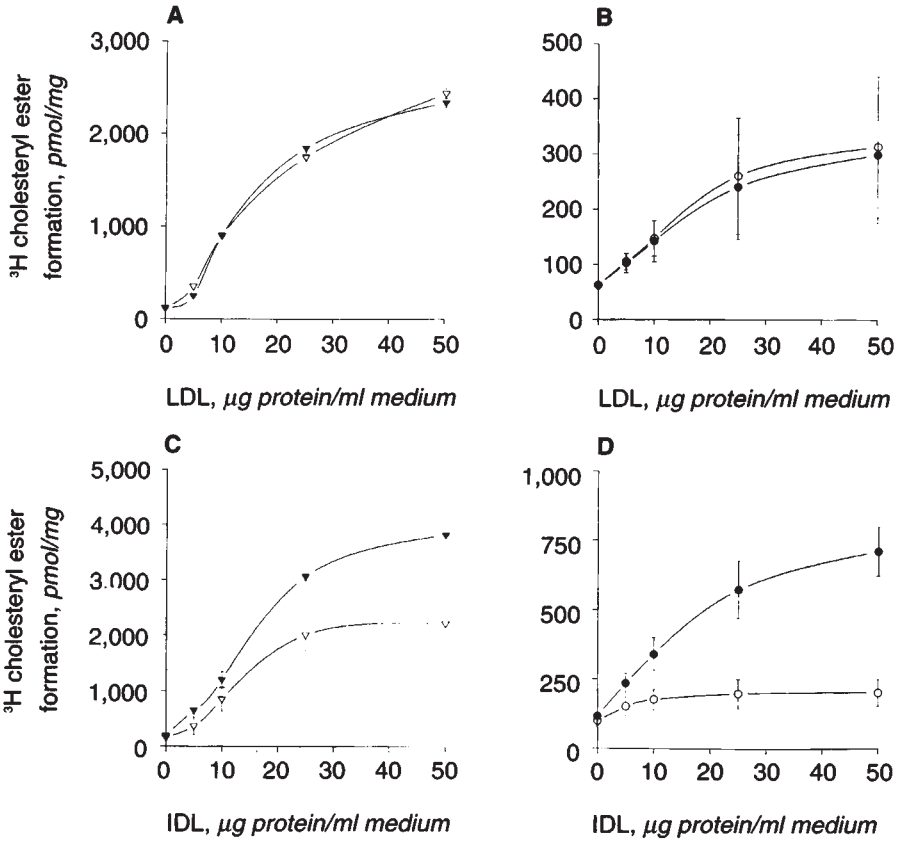

Fig. 6. *Effects of IDL and LDL from healthy subjects (open symbols) and patients with the nephrotic syndrome (closed symbols) on formation of cholesteryl esters in human skin fibroblasts (triangles) and glomerular epithelial cells (circles).* Cholesterol esterification is given as picomol [^{3}H]-oleate formed/mg cell protein/2 hr. Data are given as mean of three independent determinations. All measurements were carried out in duplicate. For symbols without error bars the SE mean was less than symbol size.

measured. In the LDL density class cholesterol, triglyceride, phospholipid and protein content was not significantly different in patients as compared to controls (Table 4). In contrast, IDL particles of nephrotic patients exhibited higher concentrations of cholesterol, whereas triglyceride content was reduced (Table 5). In particular, the elevation of cholesterol in IDL was entirely due to an increase in esterified cholesterol. Furthermore, a more pronounced apoE band could be identified by SDS-PAGE in the IDL density range of nephrotic serum (Fig. 1). In addition, determination of apoE by nephelometric methods revealed a higher concentration in the nephrotic IDL as compared to control IDL (1.5 ± 0.5 vs. 0.8 ± 0.1 mg/dl, respectively).

Uptake of lipoproteins, isolated from nephrotic patients

To determine whether receptor-mediated cellular uptake of lipoproteins from nephrotic patients was altered, various concentrations of ^{125}I labeled IDL (1 to 20 μg protein/ml) and LDL (5 to 100 μg protein/ml) were incubated with both cell lines. LDL-receptor activity was up-regulated by incubating in a medium containing 10% LDS for 48 hours.

GEC as well as fibroblasts bound, internalized and degraded normal and nephrotic LDL in a concentration and receptor dependent manner, and the process was saturable. However, GEC showed only 15% of the maximal uptake capacity as compared to fibroblasts (Figs. 2 and 3). Scatchard plot analysis revealed comparable K_d values and maximal capacities (V_{max}) for control and nephrotic LDL concerning binding, internalization and degradation in both cell lines (Table 6). Due to the high turnover rate of IDL in serum of healthy subjects, normal IDL could not be isolated from one serum sample in quantities allowing labeling of these lipoproteins with ^{125}iodine. Therefore, IDL from four apoE phenotype-matched healthy subjects was pooled. In comparison to control IDL, cholesterol-rich IDL of nephrotic patients was taken up by GEC and fibroblasts with higher affinities in a concentration dependent and saturable manner (Fig. 4). Maximal capacities for uptake of apoE-containing IDL by GEC were higher as compared to LDL uptake. The values for K_d in GEC were comparable to the values obtained for binding, internalization and degradation in cultured fibroblasts. However, the affinity of apoE-containing IDL was considerably higher compared to LDL uptake (Fig. 4, Table 6).

Effect of IDL and LDL from nephrotic patients on cellular sterol synthesis and cholesterol esterification

Incorporation of [^{14}C] acetate into sterols. To determine whether nephrotic lipoproteins influence the regulation of intracellular cholesterol metabolism, GEC and fibroblasts were incubated with varying concentrations of IDL and LDL from patients and healthy subjects. After 48 hours of incubation in 10% LDS-containing medium, cells were incubated for 16 hours with IDL or LDL. The presence of either nephrotic or normal LDL at a lipoprotein concentration of 10 μg protein/ml medium inhibited sterol synthesis by 83.7 ± 2.8 versus 81.7 ± 3.1% in fibroblasts and 58.5 ± 5.5 versus 55 ± 6.2% in GEC, respectively (Fig. 5). Sterol synthesis was more effectively suppressed by addition of nephrotic IDL as compared to control IDL. This was more effective in the low concentration

range. At concentrations above 5 μg protein/ml medium intracellular enzymatic processes were probably already saturated. Therefore, using higher lipoprotein concentrations, up to 10 μg protein/ml medium, the extent of inhibition was almost identical (Fig. 5).

[^{3}H]-oleate incorporation into cholesteryl ester. Incorporation of oleate into cholesteryl ester was used as a measure of effective cellular uptake of lipoproteins. In GEC and fibroblasts, no difference in the rate of cholesterol esterification could be observed between control and nephrotic LDL (Fig. 6). Fibroblasts showed an eightfold higher esterification rate of LDL cholesterol (at 50 μg/ml medium) than GEC. In contrast, both cell lines showed a higher cholesteryl ester formation rate when incubated with cholesterol-rich nephrotic IDL compared to control IDL (Fig. 6).

Discussion

Animal experiments have elucidated pathogenetic mechanisms underlining a potential role of lipoproteins in glomerular cell injury and progression of renal disease. Since lipoprotein metabolism is different in rodents and humans, we utilized a homologous system using human glomerular epithelial cells as well as skin fibroblasts and human lipoproteins, isolated from patients with the nephrotic syndrome, and studied receptor dependent uptake and intracellular cholesterol metabolism. The main findings were that glomerular epithelial cells in culture expressed higher affinities towards apoB,E containing IDL than apoB-100 containing LDL. The high affinity was accompanied by a more enhanced rate of intracellular cholesteryl ester formation, and suppressed sterol synthesis, after incubation with IDL from nephrotic patients than with control IDL. In contrast, nephrotic LDL showed no differences in uptake and intracellular cholesterol metabolism as compared to control LDL. These findings can be explained by the altered chemical composition and apolipoprotein distribution of intermediate density lipoproteins which accumulate in the serum of patients with the nephrotic syndrome.

Hyperlipidemia of the nephrotic syndrome can result from both increased synthesis [5] and reduced catabolism of lipoproteins [6, 7]. Reduced catabolism of triglyceride rich lipoproteins in humans is proposed to be due to a decrease in lipoprotein lipase activity, which can result in a delay in lipolysis and accumulation of remnant particles. Cholesteryl ester transfer protein (CETP) is responsible for distribution of cholesterol esters among lipoproteins. Altered composition of IDL particles with an enrichment in cholesterol esters to the expense of triglycerides, as found in the present study, could be consistent with the assumption that lipoprotein particles of nephrotic patients remain longer in the circulation than IDL of healthy subjects or that CETP activity is enhanced [33]. The high affinity to the receptor and the preferential uptake of IDL by glomerular epithelial cells could result in a down-regulation of the LDL-receptor (*in vitro* data not shown) and could therefore be one cause for accumulation of LDL. It can be speculated that both high levels of IDL as well as the prolonged circulation and exposure of renal and extrarenal tissue to these lipoproteins could result in atherosclerosis and glomerulosclerosis, which are often found in a variety of nephrotic renal diseases. Although the delayed catabolism of triglyeride-rich lipoproteins has been very well-established, much less is known about the catabolism of cholesterol and cholesterol-rich lipoproteins. LDL catabolism has been reported to be either normal [34] or reduced in nephrotic rats [35]. Extrapolating the results to humans is problematic, since LDL is not the principal cholesterol-bearing lipoprotein in rats. If we assume that receptor-mediated clearance of LDL is also delayed in humans, this could occur as a result of an abnormality in LDL receptor, or a defective LDL that binds poorly to the receptor [36]. However, in the present study the LDL of nephrotic patients did not show different receptor binding, internalization and degradation as well as intracellular cholesterol esterification and sterol synthesis as compared to LDL from healthy controls. Since LDL of nephrotic patients was comparable in lipid composition and apolipoprotein distribution to LDL from healthy controls, altered cellular uptake is not expected. It should be pointed out that in most other investigations where compositional change of the LDL particle was found, the LDL density class also comprised the IDL density range. The present data in GEC are consistent with maximal receptor-mediated uptake capacities for normal LDL reported by Gröne and coworkers [14]. In comparison to fibroblasts, their GEC cell line also exhibited uptake capacities of 10 to 15%. Furthermore, GEC also showed high affinities towards β-VLDL of cholesterol fed rabbits and chylomicron remnants, isolated from post-heparin plasma [14].

The LDL-receptor has two independent ligands, apoB-100 and apoE. In general, the presence of apoE increases the affinity of the lipoprotein to the receptor. In fact, apoB and apoE containing IDL show increased uptake in lower concentrations than only apoB-100 containing LDL. Due to the higher apoE content of nephrotic IDL this affinity was further enhanced in GEC as compared to fibroblasts when exposed to these lipoproteins. It has been reported that low density lipoprotein receptor-related protein (LRP) binds both apoB- and apoE-containing lipoprotein particles *in vitro* [37] and that LRP mediates uptake of apoE-enriched β-VLDL in fibroblasts. This raises the speculation about the presence of another receptor in GEC which may bind preferentially apoE containing lipoproteins.

In summary, nephrotic syndrome resulted in a substantial elevation of cholesterol-rich apoE containing IDL. Receptor mediated uptake in GEC of IDL from nephrotic patients was enhanced, and higher suppression of intracellular sterol synthesis and enhancement of cholesterol ester formation were found. In contrast, LDL did not exhibit qualitative abnormalities and showed normal uptake and intracellular cholesterol metabolism. IDL may therefore play the major role in glomerular lipid accumulation and development of glomerulosclerosis. It might also be of clinical value to identify those nephrotic patients who preferentially accumulate IDL.

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